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Exploring Effective Strategies for the Control of Gray Mold Disease in Tomatoes Caused by *Botrytis* using Bacterial Bioagents

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Abstract. Gray mold disease, caused by *Botrytis cinerea*, is a significant threat to tomato crops, leading to severe yield losses globally. Traditional chemical control methods pose environmental hazards and the potential for pathogen resistance, highlighting the need for sustainable alternatives. This review explores the use of bacterial bioagents as an eco-friendly and effective strategy for controlling gray mold in tomatoes. Key bacterial strains, such as *Bacillus*, *Pseudomonas*, and *Streptomyces*, have demonstrated promising biocontrol properties, including antifungal activity, competition for nutrients, and induction of plant defense mechanisms. The mechanisms through which these bacteria inhibit *Botrytis* involve the production of secondary metabolites, lytic enzymes, and volatile organic compounds (VOCs). Field trials and greenhouse studies have shown variable success, depending on environmental conditions, bioagent strain selection, and application methods. Integrated management practices combining bacterial bioagents with cultural and agronomic practices further enhance the efficacy of disease control. The study concludes that bacterial bioagents represent a viable and sustainable approach to managing gray mold in tomatoes, though further research is needed to optimize their use and scalability in commercial agriculture.

Keyword: *Botrytis*; IPM, Biological control, bacterial endophytes

Introduction

The cultivation of tomato (*Solanum lycopersicum* L.) is one of the most important horticultural and cash crops worldwide, ranking second among the crops of the family Solanaceae after potato both in area and production (Kumar et al., 2020; Anwar et al., 2019; Bauchet et al., 2012). Tomatoes represent the third most consumed vegetable (after onions and cabbage) and fifth commodity (after milk, apples, wheat, and rice) in the world. In Africa, tomato is the second most important vegetable after onion. It is grown by large and small-scale farmers both in urban and peri-urban areas for its various purposes. Global production of tomatoes in 2022 was 186.11 million metric tons (World vegetable production by type 2022 | Statista. 2024, February 6). The statistics of the Food and Agriculture Organization shows the total harvested area of tomatoes in 2022 in Asia was 269,3260 hectares and the yield was 441.546 kg/ha. Tomato is widely accepted and universally consumed due to its diverse uses. It can be consumed fresh, cooked, or in processed forms. Processed tomato products like juice, paste, sauce, and powder are often used in food preparations. Tomato is often used in daily dishes such as sauces, stews, and salads, and is a principal ingredient in many fast foods. It is a major source of vitamins and antioxidants in the diet (Beecher, 1998).

Despite the high demand for tomatoes, the production of tomatoes is lower. Diseases and pests, as well as environmental stresses, significantly affect the production and productivity of the crop (Silva, et. al. 2021; Panno, et. al. 2021). Among the diseases, gray mold caused by *Botrytis cinerea* is the most important disease of tomato (Williamson, et al., 2007). It has emerged as one of the most devastating postharvest diseases of tomato fruit. Severe gray mold outbreaks occur due to handling or packaging-related injuries, overripe fruit, or poorly ventilated storage. Once the bunches are infected, under the right temperature and relative humidity, new conidia are produced and disseminated, resulting in harvesting a single infected fruit causing widespread infection of the whole bunch. *B. cinerea* survives well on the substrate or in host plant debris, contributing to its epiphytotic nature.

Bacterial bioagents have been shown to be a potential alternative control method for several plant diseases (Tripathi et al., 2020; Tariq et al., 2020; Hussain et al., 2020). The bioagents have great potential for use as an effective control method against tomato gray mold during storage (Taha et al., 2023). This study aims to investigate effective strategies for the control of gray mold disease in tomatoes caused by *Botrytis* using bacterial bioagents.

1.1. Background and Significance

Gray mold disease, described by the ascomycete fungus *Botrytis cinerea*, is one of the most devastating diseases affecting tomato plants (*Solanum lycopersicum* L.) (Rhouma et al., 2022), leading to substantial yield losses worldwide. It can infect tomato both pre-harvest and after harvest, and it has been detected in all countries producing tomatoes, including vegetable and fruit farms, nurseries, stores and warehouses (Choquer et al., 2007). Its symptoms are grayish brown rotting lesions with a soft texture on which gray spores may appear, and it is difficult to control due to the fungus's wide host range, rapid growth, and survival structures (Holz et al., 2007). Control measures consist mainly of chemical treatments which have serious drawbacks, such as unacceptable residues on harvested produce, short harvest intervals, the emergence of resistant pathogen strains, and negative effects on human health (Ullah et al., 2024).

Plant growth-promoting rhizobacteria (PGPR) are a heterogeneous group of soil and root-associated bacteria that benefit plants and protect them from pathogens (Pandit et al., 2022; Ul Haq et al., 2024). They are found in the rhizosphere of plants and have beneficial effects on their growth, development, physiology and biomechanisms of action. They suppress nematodes and fungal, bacterial, insect and virus diseases, and many PGPRs induce systemic resistance against pathogens (Zhu et al., 2022). PGPRs reduce disease and increase growth by secreting phytohormones, showing antifungal activity, increasing soil nutrient mobilization and availability, producing antimicrobial compounds, inducing systemic resistance, promoting root development and altering biotic and abiotic stress response gene expressions in plants. PGPRs have been used successfully for the control of different diseases in various vegetable crops grown in soil or soilless substrates. (Nagrale et al., 2023)

In searching for alternative control methods for gray mold disease on tomatoes, a screening of PGPRs was carried out to evaluate their biocontrol effectiveness against *B. cinerea* on tomatoes. A total of 101 bacterial bioagents were isolated and screened. Primary screening was evaluated based on inhibit a radial growth of B. cerea mycelium on contaminated potato dextrose agar (PDA) plates. Further experiments in vitro were conducted to identify the screening isolates as potentially PGPR bacteria that produced one or more of three biocontrol mechanisms such as the production of hydrolytic enzymes, siderophores and volatile organic compounds (VOCs). In greenhouse experiments, the most promising isolates were re-evaluated based on percent diseased fruit and disease severity, and two isolates were further assessed for effective control of gray mold on tomatoes. (Imran et al., 2022)

1.2. Research Aim and Objectives

The overall goal of this research is to explore, evaluate and develop strategies for the biological control of the gray mold disease of tomatoes caused by the fungus *Botrytis cinerea*.

2. ***Botrytis*** Cinerea: The Causal Agent of Gray Mold Disease in Tomatoes

Botrytis cinerea is a necrotrophic fungus that infects pores and dead tissues of various angiosperms (Hossain et al., 2023). The fungal disease manifests as a gray-colored mold, and lifecycles of the fungi and host plants mobilize the fungi to the next growth cycle (Williamson et al., 2007). The fungus produces conidia that are carried by water droplets on the plants and germinate readily on moist leaf surfaces. Infection occurs when germ tubes penetrate wounded or senescent tissues and hyphae invade the tissue. Thereafter, the fungus invades healthy tissue causing gray mold disease (van Kan, 2003). *Botrytis* infection of crops can occur at any time from planting until harvest, although it commonly occurs just before harvest.

The plants infected often end up failing and being destroyed to avoid further contamination of the harvest. To decontaminate plants, fungicides and antibiotics can be used which limits fruit and juices development. The prevention of harvest losses by new environmentally friendly techniques is of utmost importance. Prominent alternatives to the artificial chemicals are natural toxins produced by fungi and bacteria. The toxins produced by a range of bacteria, such as antibiotics, inhibit the development of phytopathogenic fungi. Many investigations have focused on naturally occurring toxins of certain strains of *streptomycetes* and *pseudomonads* and the control of various diseases. There is, however, little or no information on the role of *Bacillus* toxins in the control of gray mold disease. (Leisen et al., 2022) (Jeblick et al., 2023)

Botrytis cinerea is a necrotrophic fungal plant pathogen with an extremely wide host spectrum that belongs to the Ascomycetes (Williamson et al., 2007; Zhong et al., 2019). Nectriaceae, the fungus is characterized by its large conidial heads formed on erect conidiophores and a greenish gray sporulating growth on a large variety of substrates (Boonmee et al., 2021). *Botrytis* produces asexual spores (conidia) that germinate under humid conditions, infecting a wide range of horticultural crops. The disease survives in ground covers as sclerotia, infecting healthy plants by offshoots of the mycelium. A wide variety of horticultural crops are attacked by *Botrytis* diseases in both sheltered and exposed situations. Infected tissues rapidly become dead, water-soaked, and sunken, and are easily crushed, revealing tawny, fluffy, and gray growths of fungal mycelium and bent conidiophores that bearing white or pale brown conidia on humid diseased surfaces. The disintegration of adjacent tissues and wilted foliage spread on both sides of the discolored patch.

Abundant conidia are discharged in dry weather to initiate further infections. The diseases may lead to the collapse of the host plant. Various flowers dropped in greenhouses and exposure to field conditions have been evaluated and the flowering response of these flowered at average greenhouse temperature and relative humidity was reduced. (Chen et al., 2022) (Bi et al., 2023)

2.1. Overview of *Botrytis* Cinerea

Botrytis cinerea is a hemibiotrophic plant pathogenic fungus belonging to the family Sclerotiniaceae (Hossain et al., 2023), known for its notorious role in the decay of a wide range of plant organs, ultimately leading to death. Commonly referred to as gray mold, it is one of the most destructive fungal pathogens. The name *Botrytis* is derived from the Latin botrus meaning “grape” and was first used by the Greek botanist Dioscorides to describe “the grape disease”. The binomial name *B. cinerea* was given in 1789 by the French botanist Jean Baptiste Pierre Antoine de Monet de Lamarck. The term “gray mold” disease is derived from the characteristic gray fungal spores that spread in the air. *B. cinerea* can infect numerous hosts in over 864 conidial hosts and 146 sclerotial hosts (Elad, et. al., 2016). The ideal environmental conditions for its spore germination, and mycelial growth are a temperature range of 20–25 °C and high humidity with water activity greater than 0.9.

Currently, tomatoes are treated with antifungal drugs, particularly synthetic fungicides, to control the disease, but the emergence of resistance in pathogens, human health problems, and environmental pollution (Goswami et al., 2018) have prompted the search for alternatives. Fresh or processed tomatoes are attacked by fungi, yeasts, and bacteria, causing spoilage by sliminess, softening, discoloration, off-flavor, and taste (Alegbeleye et al., 2022). Spoilage may also occur after prolonged refrigeration. The effects of spoilage microorganisms on tomato products may include significant economic losses to producers (Khalid et al., 2024).

Fig 1. Close-up of dichotomous branching at the tip of a condiophore with conidia of *Botrytis cinerea* from tomato. **Image credit:** Paul Bachi, University of Kentucky Research and Education Center, Bugwood.org

B. cinerea can affect plant tissues at every stage of plant development and causes post-harvest infection of fruit and vegetables, which is a major global concern in agricultural production, food industry, and health (Rhouma et al., 2023). It attacks a wide spectrum of crop plants, such as vegetables (tomato, eggplant, cabbage, etc.), fruits (strawberry, grape, peach, etc.), flowers (rose, carnation, etc.), legumes (pea, bean, etc.), tree crops (citrus, apple, etc.), and weeds (Williamson et al., 2007). Tomatoes (Solanum lycopersicum) are among the most widely cultivated vegetables in greenhouses worldwide and contribute incredibly to the economy of many countries (Padmanabhan et al., 2016). *Botrytis cinerea* causes gray mold in foliage and fruit of tomato plants and is the most economically important pathogen in tomato cultivation in the world. Even applying fungicides under protected cultivation is unfortunately not sufficient to control this disease and lost production is considerable.

While thousands of fungicides have been tested to control gray mold disease, only a few have been registered for use in tomatoes. The indiscriminate use of synthetic fungicides to control *Botrytis* diseases is not sustainable due to the rapid development of resistance to fungicides, adverse effects on bio-control agents, phytotoxicity, soil and water contamination, and negative effects on human health. Bacterial bioagents pose no phytotoxic effects on plants, humans, or the environment. The development of alternative control methods using bacterial bioagents is essential to ensuring safe and healthy production of tomatoes after the emergence of resistance (Passera et al., 2024).

2.2. Disease Cycle and Symptoms in Tomatoes

Understanding the disease cycle and symptoms caused by *Botrytis cinerea* in tomatoes is critical for effective management (SM et al., 2019). Initial infection of tomatoes by *B. cinerea* usually occurs on senescent leaves, flowers, or fruit that harbor latent (non-visible) infections (Williamson et al., 2007). Important pre-harvest factors that can lead to pre-disposal conditions of tomatoes to the infection by *B. cinerea* include mechanical injuries, malignant insect wounds, blossom drop, and senescent flower scars around fruit (Silva et al., 2023). Working with infected fruits increases the risk of secondary infections, therefore facilitating the spread and incidence of the disease in tomato crops. Pre-harvest foliar application of *B. cinerea* approved fungicides is a good pre-emptive step to avoid high disease incidence on harvested fruits. The effectiveness of such fungicide applications usually greatly decreases if application is delayed or if adverse weather conditions occur immediately after fungicide application. Other pre-harvest factors that predispose tomatoes to *B. cinerea* infection include abnormal physiological fruit ripening and senescence associated with growth disturbances or physiological alterations and high-temperature environmental conditions (24°C or above) that lead to direct sunlight and fruit sunscald (Silva et al., 2023).

Fig 2. *Botrytis* infection on tomato stem, showing brownish-gray, dry lesion and girdling of the stem. Masses of gray color *Botrytis* spores can be seen on the surface. Photo credit: *Shawn Butler, NCSU PDIC*

Fig 3. *Botrytis* infection on tomato fruit, showing rotted tissue and spores. Photo credit: *Shawn Butler, NCSU PDIC*

B. cinerea induced symptoms in tomatoes are often not visible up to one week post-infection, largely depending on the physiological and growth conditions of the tomato crop. Symptoms commonly appear on the blossom-end of the fruit as grayish-brown spots covered with fine gray mycelial growth and conidia. Initially, symptoms are coalescent, with adjacent lesions merging into large ones that rapidly expand, leading to fruit rot. The rot in fruit is soft, watery, and light brown. The conidia and hyphae of the pathogen can spread from infected fruits to adjacent and sound fruits and/or from the fruit surface to the inner tissues of the fruits by rain splash and basal cracking of the sepals. The disease can also be disseminated and transferred by contaminated equipment, workers, and containers used for harvesting and/or transporting tomatoes. (Silva et al., 2023)

3. Current Control Strategies for Gray Mold Disease

Gray mold disease, otherwise referred to as gray mold rot, is a vegetable and fruit disease which damages produce during growth, harvest, and storage (Rhouma et al., 2023). This disease is favored by high relative humidity, water film on the surface, and a temperature range between 14 and 25°C (Nakajima et al., 2014; Williamson et al., 2007). *B. cinerea* has a broad host range and frequently infects solanaceous crops like tomatoes, causing shrinkage, rottenness, and subsequent losses (Ullah et al., 2024). In crops like tomato, *B. cinerea* causes the fruit-blight disease. The pathogen sporulates profusely and kills quickly under hot (40°C) and dry (semitropical) conditions (Williamson et al., 2007).

Gray mold is a quarantine pathogen in many countries including USA, Canada, and Japan (Singh et al., 2023). Currently, vegetables and fruits from India are facing rejection due to quarantine regulation. Tomato seeds having *B. cinerea* were once intercepted in US quarantine and have posed a barrier to tomato export. *B. cinerea* has developed multi-drug resistance to benzimidazoles, dicarboximides, and sulfonamides (Shao et al., 2021). This fungus is resistant to almost all the systemic fungicides like triadimefon, myclobutanil, and azole; and STRobilurins like pyraclostrobin, phenylpyrroles etc (Rupp et al., 2017, Zhang et al., 2020). Furthermore, the increased consumer awareness for safe and environmental-friendly agriculture has discouraged the indiscriminate use of synthetic fungicides. Hence, a novel and safer control strategy is required to combat the gray mold disease.

Tomato (*Solanum lycopersicum* L.) is a major agricultural vegetable crop cultivated in tropical countries. Rotted tomato fruits are marred with conspicuous grayish brown-to-gray mold growth which is eventually covered by velvety dark brown mycelium of *B. cinerea*. This disease is the second most important quarantine problem for tomato vegetable. There are mainly two control strategies against tomato gray mold disease: chemical fungicides and biocontrol agents, both of which are being used worldwide. Persistent residues of synthetic fungicides lead to environmental and human health hazards which necessitated the development of biocontrol options against this demataceous necrotroph. (Ullah et al., 2024)

3.1. Chemical Control Methods

Chemical control methods have been widely used by tomato growers to manage *Botrytis* gray mold (Rhouma et al., 2023). The first chemical control against gray mold in tomato was the application of fungicides, usually either a preventive application or curative after infection. The first widely used fungicide, the Bordeaux mixture, was accidentally discovered by French botanist Millardet in 1885 (Millardet, 1933). The systemic fungicides were first used in the Netherlands in the mid-1970s and are still the most used control method in the Netherlands (Brent, 2012). However, there is public concern over the application of these potentially hazardous chemicals, and there is mounting visible evidence that these chemicals may be ineffective due to the development of fungicide-resistant strains of *B. cinerea*. Early reports of reduced sensitivity to benomyl and thiophanate methyl among strains of *B. cinerea* in the Netherlands were in the 1980s, because of repeatedly spraying with these fungicides (Grindle, 1981). Later, strains of *B. cinerea* with resistance to other fungicides, such as iprodione, procymidone, cyprodinil, and triadimenol, were also detected (Leroux et al., 2002).

Research on the chemical control of gray mold began in different regions worldwide, with a focus on determining selective fungicides that did not adversely affect plant growth and were effective in disease control (Kim et al., 2016). Fungicides currently in use include the dicarboximide group such as iprodione, procymidone, and the anilinopyrimidine fungicide, cyprodinil (Leroux et al., 2002). There are some fungicides such as myclobutanil, azoxystrobin, triadimefon, and probenazole showed a promise in managing *B. cinerea* on tomato. The efficacy of some compounds among the registered fungicides in Egypt for the control of gray mold disease in field conditions was tested (Dib et al., 2017). In Italy, effectiveness of four synthetic fungicides has been studied in the control of post-harvest gray mold of strawberry (Vischetti et al., 2023). There are many newer fungicides available, but few have been tested against gray mold in tomato.

The chemical control practices employed to control gray mold in the different greenhouse production areas, control points, and recent advances made in practicing the chemical control of this economic threat to the tomato industry of the Philippines were reviewed. A long-term field experiment was conducted to determine a sustainable strategy for producing dry beans with no or minimal tillage, with the use of soil-incorporated herbicides and pre-emergence applied fungicides; trials were done in 2010, 2011, and 2013. Two bacterial bioagents, *Bacillus licheniformis* and

Pseudomonas fluorescens, used either alone or in combination have the potential to control gray mold in tomato flowers. Furthermore, if used as preventive agents, CAL, HAL, and KAL have the potential to control gray mold in tomato production. (Taha et al. 2023)

3.2. Biological Control Methods

There is increasing interest in alternative strategies for controlling postharvest decay that minimize the reliance on chemicals and contribute to the goal of sustainable agriculture. In this context, biocontrol offers an interesting alternative that has been successful at various levels (Pandit et al., 2022). Certain bacteria from the genus *Pseudomonas* and species of *Bacillus* producing lytic enzymes have been shown to suppress numerous pathogens in vitro and in vivo. In addition, several bacterial strains have been proposed as biostimulants in agriculture, showcasing beneficial effects on different kinds of crops. The potential of two *Bacillus spp.* as biocontrol agents against gray mold decay on tomatoes was evaluated in the light of the mode of action involved in the control and regarding their ability to promote growth. (Lyng et al., 2024)

Postharvest diseases caused by fungi represent a significant problem in horticulture that can lead to major economic losses due to the decay of harvested products. Gray mold, caused by *Botrytis cinerea*, occurs on a wide range of horticultural and fruit crops and is responsible for large quantitative losses worldwide (Ullah et al., 2024). Currently, this disease is mainly controlled by fungicide application, although the effectiveness of such control measures is under continuous scrutiny and has even been questioned after the detection of resistant strains of the pathogen. In addition, regulations on the use of pesticides on commodities for human consumption are becoming stricter in many countries. On the contrary, consumers and marketing organizations are demanding safer food, with minimal pesticide residues or no pesticides. Consequently, there is an increasing interest in alternative control strategies (Ab Rahman et al., 2018) that either have little or no impact on the environment and human health.

Biocontrol with antagonistic bacteria could be one of the most sustainable strategies for managing gray mold disease and other postharvest decays (Haq et al., 2024). Specific *Pseudomonas* and *Bacillus spp.* were shown to exert a significant and consistent reduction of gray mold on tomatoes and other commodities, establishing a good basis for future work aimed at improving the efficiency of these bioprotectants for commercial purposes. In plant systems, postharvest biocontrol is usually less effective than preharvest biocontrol, possibly due to the lack of colonization and a subsequent depletion of the biocontrol agents.

4. Bacterial Bioagents as Promising Alternatives

Global agriculture faces significant threats due to the junction of rising population and food security with the effects of climate change (Saleem et al., 2024; Mahato, 2014; Howden et al., 2007). This paradigm shift is complemented by an upsurge of agricultural pathogens that are invading crop fields, limiting crop productivity. In this perspective, an overview of the effectiveness of bacterial bioagents as alternatives to chemical fungicides in combating gray mold and other fungal pathogens in tomato is presented.

4.1. Overview of Bacterial Bioagents

Gray mold disease, caused by the necrotrophic fungus *Botrytis cinerea*, is one of the oldest and most widely studied diseases in the world (Williamson, et. al., 2007., Dean et al., 2012). There is also increasing public concern regarding the environmental effects of synthetic fungicides (Geiger et al., 2010; Muñoz-Leoz et al., 2011). Consequently, efforts are ongoing to shift from synthetic fungicides to bioprotectants to minimize the impacts of chemicals on human health and the environment. In recent years, there has been a growing interest in the use of bacterial bioagents as alternate biocontrol agents to fungicides. Bacterial bioagents are generally antagonistic plant-growth-promoting bacteria (PGPB) or endophytes belonging to genera *Pseudomonas*, *Bacillus*, *Burkholderia*, etc. Bioagents can combat multiple pathogens, including bacterial, viruses, and fungi. Bacterial bioagents can inhabit a variety of niches in plants, enabling them to exert biocontrol efficacy at multiple sites simultaneously. Bacterial bioagents have been demonstrated to eradicate *B. cinerea* at pre- and post-harvest levels in several fruits and vegetables, including tomatoes. In greenhouse experiments, the Gram-positive species *B. amyloliquefaciens* and *B. subtilis* reduced the incidence of gray mold disease on attached fruits in the vine, which was more effective than chemicals (Lastochkina et al., 2019; Zhou et al., 2020). *B. subtilis* was the only bioagent that countered *B. cinerea* in detached-vine tomatoes under in vitro conditions. *B. amyloliquefaciens*, a *Bacillus* species, produced antifungal compounds such as surfactin, iturin, and fengycin, leading to inhibition of gray mold in tomatoes and strawberries (Imran et al., 2022).

Table 1. List of bacterial agents for controlling *Botrytis cinerea* through the production of diffusible antifungal metabolites, phytohormones, and plant defense enzymes.

Bacterial agent	Condition	Mechanism of action	Antagonistic Effect	Reference
Pseudomonas aeruginosa CQ-40	In vitro, Tomato plants, seeds, fruits	Solubilize phosphorus, fix nitrogen, and secondary metabolites	Induction of systemic resistance (ISR)	Wang et al., 2020
Trichoderma harzianum	In vitro and greenhouse	Antifungal metabolites	Induction of systemic resistance (ISR)	Imran et al., 2022
Bacillus subtilis L1-21	In vitro, tomato fruits	Antifungal metabolites	Mycelial growth inhibition	Bu et al., 2021
B. amyloliquefaciens strains BBC023 and BBC047	Roots and leaves of tomato plants	Production of indole acetic acid (IAA) and 2,3-butanediol, generate robust biofilms and colonize the phylloplane	Induction of systemic resistance (ISR)	Salvatierra-Martinez et al., 2018
Pseudomonas aeruginosa 7NSK2	Tomato plants and *Arabidopsis thaliana*	Pyochelin and pyocyanin	Induction of systemic resistance (ISR)	Aznar & Dellagi, 2015
Enterobacter cowanii B-6-1	Tomato fruits	Antifungal metabolites	In vitro inhibitory effect	Shi & Sun, 2017

Pseudomonas antimicrobica	In vitro	Antifungal metabolites	Inhibit germination of conidia	Innes & Allan, 2001
Actinoalloteichus cyanogriseus 12A22	In vitro	2-Hydroxyethyl-3-methyl-1,4-naphthoquinone	Mycelial growth inhibition	Zhang et al., 2021
B. velezensis strains 5YN8 and DSN012	Pepper plants	Secondary metabolites and volatile organic compounds (VOCs)	Induction of systemic resistance (ISR)	Jiang et al., 2018
Pseudomonas aeruginosa LV strain	In vitro	Phenazine-1-carboxylic acid (PCA)	Mycelial growth inhibition	Simionato et al., 2017
Bacillus subtilis NCD-2	Apple fruit	Fengycin	Open pores in the plasma membrane	Su et al., 2020
Bacillus velezensis Bvel1	Pepper and grape plants	Bacillibactin	Suppression of fungal growth by chelating the available ferric iron	Nifakos et al., 2021
Bacillus velezensis XT1	In vitro and in fruits	Surfactin, fengycin, and bacillomycin	Open pores in the plasma membrane	Toral et al., 2018
Kosakonia radicincitans DSM 16656	In vitro and in apple fruit	Enterochelin	Blocking the polygalacturonase	Lambrese et al., 2018
Ochrobactrum cicero MM17	In vitro and *Lilium* L.	Propanoic acid, −hydoxy-methyl ester; phthalic acid, hex-3-yl isobutyl ester and phthalic acid, hept-3-yl isobutyl ester	Suppression of mycelial growth	Priyanka & Nakkeeran, 2019
Pantoea sp. MQT16M1	In vitro and in strawberry fruits	Salicylamide, maculosin, and herniarin	Disruption of cell wall components	de Moura et al., 2021
Bacillus amyloliquefaciens VB7	In vitro and foliar application	Phthalic acid, hept-3-yl isobutyl ester and propanoic acid,2-hydroxy-, methyl ester	Mycelial growth inhibition	Barka et al., 2000
Bacillus cereus strain B-02	In vitro	Bacteriocins, toxins and other metabolites	Act on DNA and mitochondrion	Li et at., 2012
Bacillus amyloliquefaciens	In vitro and foliar application	Solubilize phosphorus and zinc as well as IAC production	Induction of systemic resistance (ISR) and Mycelial growth inhibition	Rahman et at., 2025

There are some bacterial genera such as *Pseudomonas*, *LactoBacillus*, *Propionibacterium*, *Microbacterium*, and *Corynebacterium* have been isolated from spoiled tomatoes (Obafemi et al., 2019; Ghosh, 2009; Sola et al., 2022). The spoilage activity of these bacteria has been associated with the enzymatic production of exopolysaccharides (Iosca et al., 2022; Guérin et al., 2020). Bioagents are living organisms with the capacity to suppress the infection and growth of phytopathogenic microorganisms in plants and environmental substrates. The most extensively investigated bioagents have been bacteria, yeast, and fungi. *Bacillus* and *Pseudomonas* species are the most studied bacteria groups with potential as biocontrol agents due to their ability to suppress a broad spectrum of fungal and bacterial pathogens (Balthazar et al., 2022; Lyng et al., 2024)).

Pseudomonas fluorescens is a well-known species in agricultural fields (Hol et al., 2013), but *B. subtilis* has also shown antifungal activity against various phytopathogenic fungi (Mardanova et al., 2016). In recent years, other *Bacillus* species have been studied, but there is a lack of knowledge regarding the biocontrol potential of other bacterial genera and species.

4.2. Mechanisms of Action

Bacterial agents can combat pathogens by using multiple mechanisms, such as production of antifungal compounds, induction of systemic resistance, competition for nutrients and space, and enhancement of plant growth (Bonaterra et al., 2022; Köhl et al., 2019). Synthetic fungicides can effectively eliminate fungal pathogens, but extensive use of fungicides often creates resistant strains, leading to failure in disease control. Unlike fungicides, bacterial bioagents with plant-growth-promoting bacteria are safer and have many modes of action against fungal pathogens (Ngalimat et al., 2021; Vílchez et al., 2016). Plant growth promotion can be accomplished by phosphate solubilization, nitrogen fixation, indole acetic acid (IAA) production, and siderophore synthesis, promoting root and shoot growth under normal and stress conditions by directly affecting plant hormones. There is ongoing research to understand the mechanisms of bioagents, including in vitro antifungal activity, green house, and field studies on bioprotection efficacy against *B. cinerea* and other pathogens. Understanding the role of individual compounds in biocontrol and elucidating the underlying mechanisms will pave the way to enhance bioagent effectiveness, and subsequent products may contain a cocktail of suitable strains or purified compounds to achieve desired efficacy against specific pathogens (Tripathi et al., 2020).

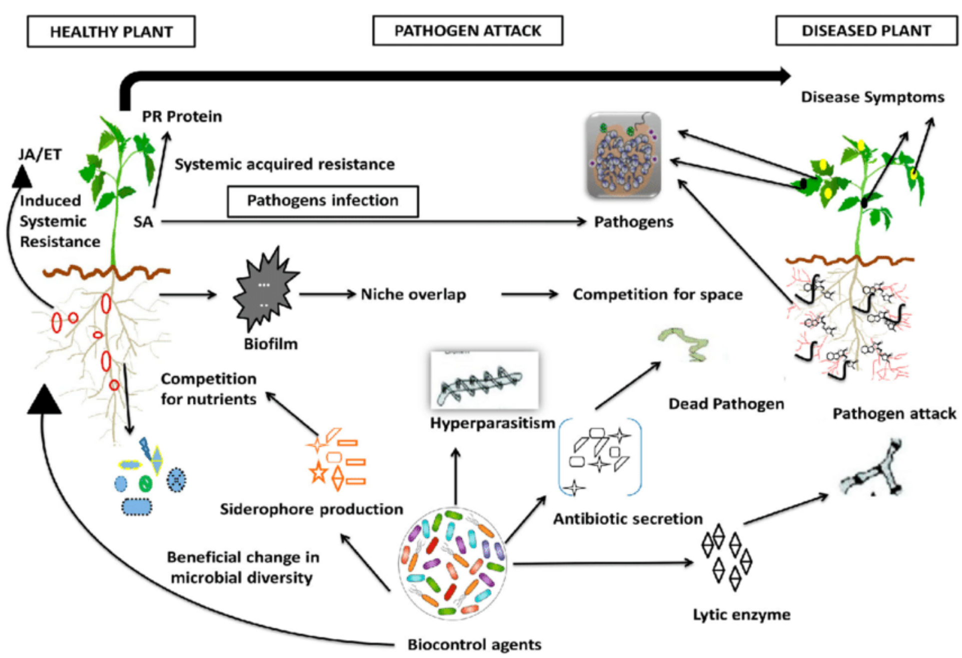

Fig 4. Mechanism of biocontrol agent against pathogenic microorganism (Tariq et al., 2020)

Bacterial bioagents have emerged as frontline biocontrol agents against a spectrum of fungal pathogens, acting via a multi-faceted approach that could be broadly classified into four distinct mechanisms: (1) direct antagonism of the pathogen, (2) competition for resources, (3) induction of plant defense response, and (4) alteration of environmental conditions to disadvantage the pathogen.

1. Direct Antagonism of the Pathogen: Bacterial bioagents secrete inhibitory compounds such as antibiotics, lytic enzymes, and volatile organic compounds (VOCs) to mount a direct attack against the pathogen (Singh et al., 2023). The spectrum of efficacy of bacterial bioagents depends on the intensity and the efficacy of the inhibition of the pathogen growth. Three putative antifungal metabolites, i.e., pyoluteorin, 2,4-diacetylphloroglucinol, and harzianopyridone, were found to efficiently inhibit mycelial growth of *B. cinerea* (Balthazar et al., 2022). More than 20 antifungal compounds were reported from various bacterial bioagents (Brauer et al., 2019). These may broadly include lytic enzymes, such as chitinases and β-1,3-glucanases, that degrade the structural components of the fungal cell wall. Treatment of *B. cinerea* with subtilisin or chitosanase, or a combination of both, resulted in the formation of protoplasts and led to a complete loss of infectivity (Liu et al., 2020). Lipopeptide surfactin produced by *Bacillus subtilis* B26 was also demonstrated to induce the lysis of the pathogen hyphae (Kourmentza et al., 2021).

2. Competition for Resources: Bacterial bioagents compete with the pathogen for nutritional or ecological requirements such as space, nutrients, and moisture. Siderophores are important bacterial metabolites that chelate ferric ions thereby reducing the bioavailability of iron available to the pathogen in a given ecological niche. Siderophore-producing bacterial bioagents effectively suppressed the growth of gray mold in in vitro bioassays (Timofeeva et al., 2022). Suppression of other fungal phytopathogens by bacterial bioagents was also reported via the mechanism of competition for iron using hydroxamates produced by bacteria in the rhizosphere. (El-Saadony et al., 2022; Abdelhalim et al., 2024)

3. Induction of Plant Defense Response: Lysates, culture filtrates, or intact cells of bacterial bioagents are known to trigger systemic resistance against various fungal, bacterial, and viral pathogens in disease-susceptible crop cultivars. Such immune responses include the upregulation of specific genes associated with the synthesis of phenylpropanoids, salicylic acid, jasmonate, and ethylene, leading to the accumulation of PR proteins and phytoalexins. (Meena et al., 2022)

4. Alteration of Environmental Conditions: Several bacterial bioagents are known to alter the environmental conditions and biochemical indices that may cause an unfavorable environment for the pathogen. These include lowering pH, reducing moisture, the production of volatile compounds, or increasing the relative humidity that could be detrimental to the pathogen physiology. Induced resistance against anthracnose in papaya fruits was reported following treatments with volatile compounds, such as acetic acid, 3-methylbutanol, and isoprene, derived from *Pseudomonas fluorescens*. (El-Saadony et al., 2022)

5. Challenges and Future Directions

5. Challenges and Future Directions

5.1. Challenges in Implementing Bacterial Bioagents

While the use of bacterial bioagents holds great promise in the biocontrol of gray mold disease (Elnahal et al., 2022; Khan et al., 2022), there are several challenges that must be addressed for successful implementation. First, the bioagents need to be compatible with the existing agricultural practices. Many farmers rely on chemical fungicides for the control of gray mold disease, but these chemicals may also have negative effects on the bioagents. Therefore, tests need to be conducted to ensure that the bacterial bioagents are not inhibited by commonly used fungicides. Second, the efficacy of the bioagents may vary under different environmental conditions (Larkin & Fravel, 2002; Abd-Elgawad & Askary, 2020). Temperature, humidity, and light conditions can all affect the viability and activity of the bioagents. To address this challenge, experiments should investigate the performance of the bioagents under different conditions and identify the conditions under which they are most effective.

5.2. Future Research Directions

To fully realize the potential of bacterial bioagents in the biocontrol of gray mold disease, several areas of future research need to be prioritized. First, more efforts should be directed towards isolating and characterizing novel strains of bacteria with strong biocontrol activity (Lian et al., 2022). The focus should be on bacteria that have been isolated from plant rhizosphere or leaf surfaces, as these bacteria are more likely to possess traits that promote colonization and survival in plant environments. Second, studies should investigate the mechanisms of action of the bacterial bioagents at the molecular level (Köhl et al., 2019). Understanding the mechanisms of action can provide insights into how to enhance the efficacy of the bioagents and can also help in the selection of appropriate bioagents for different plant-pathogen systems. Lastly, economic evaluations of the bacterial bioagents (Price et al., 2023) should be conducted to assess the feasibility of their commercial production and application in the biocontrol of gray mold disease. Studies should investigate the cost-effectiveness of the bacterial bioagents compared to traditional fungicides and identify potential market opportunities for the bioagents (Fenta & Mekonnen, 2024). Addressing these challenges and pursuing these future research directions can facilitate the adoption of bacterial bioagents in agriculture and contribute to sustainable food production systems.

5.1. Challenges in Implementing Bacterial Bioagents

Recent biological control research, experiments and field trials using different bacterial bioagents have been carried out to explore the possibilities of their use for controlling gray mold disease of tomatoes caused by *Botrytis* (Bonaterra et al., 2022, Ayaz et al., 2023). Several trap bacteria found effective in dual culture bioassays were selected for use against the target fungal pathogen in challenge plate bioassays (Niño-Sánchez et al., 2021). They were found effective by producing different types of mycoparasitic structures like appressoria, lobate hyphal coils, and oozing hyphal coils (Hasan et al., 2022). Effects of these bioagents were assessed on host plant growth promotion by measuring various plant growth parameters like root and shoot length, number of leaves and flowering, and fruit yield (Imran et al., 2023). Notable improvements in plant growth parameters were observed, which varied from one bioagent to another. Mass multiplication of the best bacterial bioagent was done on different media, and its efficacy was tested on the tomato crop under natural conditions of field and polyhouse. The bioagent gave good control of gray mold disease droplets of *B. cinerea*, which is still a matter of uncertainty.

Challenges in adopting fungal or bacterial bioagents for the biological control of postharvest diseases of fruits and vegetables exist at key levels of bioagent development (Abd-Elgawad & Askary, 2020). Critical issues in terms of aging and loss of viability in storage, which are vital to bioagent usefulness, need to be addressed (Berninger et al., 2018). Showering research on this topic is expected to enhance the smooth integration of bioagents in the mainstream fruit and vegetable postharvest industry. Some factors influencing its efficacy were found in the following signs. Many of these abiotic factors were shown to increase the incidence of diseases. There are some other advantages of using multiple bioagents. Most of the studies regarding biocontrol agents have been concentrated on the in vitro or lab level or very few have been reported at the preharvest level. They also need validation at a more practical level. Not all environments are favorable for all agents. Interactions between bioagents and environmental conditions need to be explored for a better understanding of using multiple bioagents. The advantage or disadvantage of a bioagent in one situation may differ under other conditions. It requires gainful compromise and detailed studies on each pathogen and its agenda and bioagent. (El-Saadony et al., 2022) (Palmieri et al., 2022)

5.2. Future Research Directions

Moving forward, it is essential to continue exploring effective bacterial bioagents that can control gray mold disease in tomatoes. Screening effective strains using the in vitro assessment technique is an essential first step in developing biocontrol agents. By analyzing the potential bacterial isolates in controlling *Botrytis* disease in tomatoes, it is easier to identify and select bacterial bioagents that may yield promising results in the field (Akça & Tozlu, 2022; Imran et al., 2023). This research can be applied to other crops similarly infected by *Botrytis* species. Addressing the research gap on effective bacterial bioagents for controlling *Botrytis* diseases in tomatoes and other crops will positively affect economic development and food security.

While recent studies concentrated on new strains of the Gluconobacter genus from the IBBR strain collection, in the future, it may be beneficial to search for additional bioagents of different genera (Purushotham et al., 2024). In addition, screening the agar containing the bacterial strain and tomato fruit may be affected by various environmental conditions. Pest management in practice should involve considering the location, specific crop varieties, and pest conditions. Testing new bioagents under diverse conditions is necessary to identify and select bioagents adaptable to various environmental conditions (Köhl et. al., 2011), allowing them to flourish in fields where the bioagents are deployed. Movement experiments of the bioagents may also enhance efficacy, as it is likely that agents that drift on the wind could effectively colonize new crops and environments, enhancing the chances of dependent viruses being transmitted. This may be a beneficial direction for future research. In addition, research into whether bacteria can induce resistance would be a beneficial step in understanding interactions with pathogens. Inducing resistance with an emphasis on boosting natural resistance mechanisms could provide a safe and effective means of pest management.

Future research should also prioritize determining the impact of the selected bioagent strains on non-target organisms, such as beneficial soil bacteria, insects, and macro-organisms (Vanama et al., 2023). Understanding the effects on beneficial microbes, including food webs in and above soil, will be beneficial to biocontrol. Macro-organisms such as predators and parasitoids that prey on target insects are essential components of integrated pest management (Bellows, & Fisher, 1999; Lenteren, 2003) that need to be considered to prevent unexpected outcomes. The environmental fate and activity of the bacterial bioagents should also be assessed, as it is unknown how long post-application the strains will remain viable and active after being introduced to a new environment.

6. Conclusion and Implications for Agriculture

In recent years, various studies have demonstrated the potential of bacterial antagonists to manage plant diseases caused by phytopathogenic fungi (Ayaz et al., 2023, El-Saadony et al., 2022). The present investigation dealt with the screening of various bacterial bioagents for their efficacy against gray mold disease of tomato, caused by *Botrytis* cinerea.

Bacterial bioagents could play a significant role as an organic alternative to synthetic chemical fungicide in the integrated disease management strategy against gray mold disease in tomato. The field application of biocontrol agents has also been discussed. Thus, the present study demonstrates the potential of bacterial bioagents for the control of gray mold disease in tomato caused by *Botrytis cinerea*. There is a dire need to address the issue of food security of world since farming is the mainstay for livelihood for many parts of world (Giller et al., 2021). However, modern agriculture, based on monocultures and heavy use of fertilizers and pesticides, has resulted in stagnated yields over time, making the food production system fragile, extremely vendor driven and unsustainable (Weis, 2007). There is urgent need to find alternative options especially for vegetable crops grown in protected environments such as greenhouses, polyhouses, tunnels since failure of vegetable crops would severely affect the livelihood of the farmers.

World tomato production reached to nearly 186.82 million tons from total area of 5 million hectare with an average productivity of 36.97 t/ha (FAOSTAT 2022). Tomato production is plagued by various diseases caused by necrotrophic fungal pathogens *Botrytis cinerea* is of economic importance especially in humid and wet weather condition with severe postharvest losses due to rotting of fruits. Synthetic chemical fungicides are not only carcinogenic but also result in the development of fungicide resistant pathogens (Goswami et al., 2018, Ul Haq et al., 2020). Keeping all these threats in mind, the present investigation was undertaken with the aim to explore the potential bacteria-based biocontrol agents to manage the diseases of tomato under different cropping situations within the ambit of sustainable agriculture.

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استكشاف استراتيجيات فعّالة لمكافحة مرض العفن الرمادي في الطماطم الناتج عن فطر البوتريتيس باستخدام العوامل الحيوية البكتيرية

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مستخلص. يشكل مرض العفن الرمادي، الناتج عن فطر البوتريتيس سينيريا، تهديدًا كبيرًا لمحاصيل الطماطم، مما يؤدي إلى خسائر فادحة في المحصول على مستوى العالم. وتشكل طرق المكافحة الكيميائية التقليدية مخاطر بيئية وإمكانية مقاومة مسببات الأمراض، مما يسلط الضوء على الحاجة إلى بدائل مستدامة. يستكشف هذا الاستعراض استخدام العوامل الحيوية البكتيرية كاستراتيجية صديقة للبيئة وفعالة لمكافحة العفن الرمادي في الطماطم. أظهرت سلالات بكتيرية رئيسية، مثل *Bacillus* و*Pseudomonas* وStreptomyces، خصائص واعدة في مجال المكافحة الحيوية، بما في ذلك النشاط المضاد للفطريات، والتنافس على العناصر الغذائية، وتحفيز آليات الدفاع النباتية. تتضمن الآليات التي تمنع بها هذه البكتيريا العفن الرمادي إنتاج المستقلبات الثانوية والإنزيمات التحللية والمركبات العضوية المتطايرة (VOCs). أظهرت التجارب الميدانية ودراسات البيوت الزجاجية نجاحًا متفاوتًا، اعتمادًا على الظروف البيئية واختيار سلالة العامل الحيوي وطرق التطبيق. تعمل ممارسات الإدارة المتكاملة التي تجمع بين العوامل الحيوية البكتيرية والممارسات الثقافية والزراعية على تعزيز فعالية مكافحة الأمراض. وخلصت الدراسة إلى أن العوامل الحيوية البكتيرية تمثل نهجًا قابلاً للتطبيق ومستدامًا لإدارة العفن الرمادي في الطماطم، على الرغم من الحاجة إلى مزيد من البحث لتحسين استخدامها وقابليتها للتوسع في الزراعة التجارية.

الكلمات المفتاحية: العفن الرمادي؛ الإدارة المتكاملة للآفات، المكافحة الحيوية، الفطريات البكتيرية الداخلية